REMARKS

1. Based on the Office Action and Office communication, the applicants have amended the claim 1 to overcome the claim objection. The Claim 1 has been amended to add the limitation of "pores are about 0.4 to 40 microns in diameter."

2. The pore size is first limited to "sufficient for separating mesenchymal stem cells from other cells".[0025] During that time, people assaying migration of cells like haematopoietic stem cells frequently used the commercial product (Transwell^{R}; Corning Inc.; Corning, NY; http://www.corning.com) which had pores with size at 0.4, 3, 5, 8 and 11 μm. We also used the devices with these different sized pores in this application, and found all of them worked. Part of the result was presented n in the paper published in **Stem Cells** 20:249-258, 2002 with the pore size to be 3 μm. Furthermore, MSCs was known to be spindle like with one diameter larger than 50 μm. The upper limit was therefore set at 40 μm. Thus, we limited the pore size range from about 0.4 to 40 μm.

   Further the Transwell devices have long been used for migration assay for a long time, cells smaller than the size will migrate through the pores. The claim said "sufficient for separating mesenchymal stem cells from other cells (e.g. haematopoietic stem cells". The "haematopoietic stem cells" was only an example. We included several papers published before 2000 to support this point.

3. Prockop et al showed RS cells can be separated from other mMSCs by ultrafiltration through an ultrafiltration membrane having appropriately-sized pores (e.g. 10 μm). However the example was done using **a frozen stock of MSCs**, which did not contain haematopoietic stem cells. Therefore, the cells in the lower well were not CD34+. Aside from, some haematopoietic stem cells or progenitor of haematopoietic stem cells are CD34-. Thus, CD34 is not a good marker of haematopoietic stem cells.

   The Goodell et al.'s reference (1997, Nat. Med. 12:1337-1345) cited in Prockop et al.'s US patent No. 7,374,937B1 mentioned that "SP cells were originally identified in marrow as rare cells that are **small in size** and rapidly secrete a series of labeling dyes, because they contain a large amount of a multi-drug-resistant protein. SP cells are **CD34-negative**, but are **precursors of CD34-positive hematopoietic stem cells and other hematopoietic cells.**"

This specification described the upper plate with pores, wherein the pore size is "sufficient for separating mesenchymal stem cells from other cells (e.g. haematopoietic stem cells". It would not be important that the cells passed through the pore were CD34+ or CD34- haematopoietic stem cells.

References to show transwell used for CD34+ or other haematopoietic cell migration:

a. 1999, Blood, 94:509-518.

In vitro chemotaxis of WAS neutrophils and monocytes has been shown to be deficient.[3,41,42] It was recently reported that SDF-1a could induce the migration of MKs through endothelial cells43 or directly through a 5-μm pore size transwell.[44,45]

Migration assays were performed using 5-μm pore filters (**Transwell**, 24-well cell clusters; Costar)

b. 1999, J. Exp. Med., 190:1755-1768.

Immediately thereafter, Costar transwell devices with 5-μm pore size, polyvinylpyrrolidone-free polycarbonate membranes were inserted into the wells, and 100 μl cell suspension was layered on top of the membrane.

c. 1999, Blood, 93:1511-1523.

The migration assay was performed using 5-μm or 8-μm pore filters (**Transwell**, 24-well cell clusters; Costar, Cambridge, MA)

d. 1998, J. Immunol., 160:2418-2424.

5 x 10 5 peripheral blood cells after RBC lysis were added to the upper chamber of Costar **Transwell** (6.5 mm diameter, 3 μm **pore size**, polycarbonate membrane

e. 1999, Mol. Cell. Biol., 19:7473–7480.

The membranes of **transwell** chambers (**8-μm-pore-size** polycarbonate membrane ...

f. 1997, Blood, 89:1165-1172.

CD34 + bone marrow cells (1 × 10 5) were seeded into the top chamber of **TransWell** (Corning Costar Corp) dual-chambered 24-well plates (0.4-μm **pore size**) with 0.7 mL total of a 1:1 mixture of DMEM/F12 medium

supplemented with 10% fetal calf serum,

4. This application first combined the biological and physical characteristics of MSCs to provide an isolated and culturing population of MSCs. Based on this application, those with ordinary skill in the art were able to make and/or use the claimed invention without undue amount of experimentation.

Accordingly, this application should be placed in condition of allowance. An early Notice to this effect is respectfully expected.

Respectfully submitted:
By: Shih-Chieh Hung

Shih-Chieh Hung
886-2-28757557 ext 118 (voice)
886-2-28265164 (fax)
hungsc@vghtpe.gov.tw(email)
No 201 Shih-Pai Rd Section II,
Taipei-Veterans General Hospital, Taipei,
Taiwan, R. O. C.